-25- (Amended)

The method of Claim 19 wherein the removed [separated] proteins have been precipitated together from a combined mixture of the first and second supernatants [combined together] using acetone and then isolated and dispensed in sterile distilled water to provide the vaccine.

-26- (New)

The method of Claim 16 wherein the mixed intracellular proteins and mixed extracellular proteins in water have been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine.

-27- (New)

The method of Claim 18 wherein the mixed intracellular proteins and mixed extracellular proteins in water has been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine.

REMARKS

Claims 16 to 27 are pending. Claims 26 and 27 are new. No claims are allowed.

1. The specification was objected to under 35 USC 112, first paragraph, as requiring a deposit. Enclosed is a Declaration by the attorney of record indicating that the deposit has been made under the terms

of the Budapest Treaty. This Declaration states in paragraph 6:

> "[t]hat, with respect to availability of the culture(s), I state that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if a patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent (M.P.E.P. § 608.01(p))...."

This should complete the requirements for a biological deposit. An acknowledgment of this deposit has been amended into the specification on page 5, lines 21-24. In addition, on page 5, line 25 of the specification, the address for the American Type Culture Collection has been corrected.

2. The specification and Claim 22 have been corrected as to "Sabouraud's". This is not a trademark, but rather the name of the person who developed it.

3. Claims 16 to 25 were rejected under 35 USC 112, first paragraph, as containing subject matter not enabled by the specification. The discussion at page 6, line 15 is only the preamble to the following Example 1. The disclosure in Example 1, paragraph 7 is a test to verify that the principal proteins are present and is not a separation step. The use of thimersol in Example 1 is a well known technique which makes certain there are no viable cells in the vaccine.

The Mendoza (IDS-AF (1992)) publication describes the Miller et al (1985) vaccine which is the cell mass vaccine (CMV) (mixed intracellular proteins).

The soluble concentrated antigen vaccine (SCAV) contains the mixed extracellular proteins prepared by Dr. Mendoza in 1992. The vaccine used in the present method contains both the CMV and SCAV proteins (page 5, lines 4 to 12 of the specification and Claims 16 and 18). Miller et al (1981) (reference of record) describes the CMV vaccine in detail at page 378.

It is agreed that the prior art does not describe a method for preparing a vaccine involving mixed extracellular and mixed intracellular proteins. The Applicant does not have to know the "structure" of the proteins, only that the vaccine with the combined mixed extracellular and intracellular proteins are effective as shown in the specification. The vaccine is the combination of the separate mixed proteins. Clearly the claims are enabled by Example 1 and the rest of the specification. Reconsideration of this rejection is requested.

4. Claims 16, 18 and 19 were rejected under 35 USC 112, second paragraph. These claims have been modified to clearly reflect the vaccine as described in Example 1. Reconsideration is requested.

5. Claims 18, 20-22 and 24 were rejected under 35 USC 102(b) as being anticipated by Mendoza et al (IDS-AI; 1992). As discussed above, Mendoza et al (AI) teaches two separate methods for producing *Pythium insidiosum* vaccines, a cell-mass vaccine (CMV) and a soluble concentrated antigen vaccine (SCAV). Both

vaccines were shown to be useful as immunotherapy vaccines to cure horses infected with *P. insidiosum* in less than 0.5 months. The vaccines were of limited value for treating horses infected greater than 0.5 months but less than 2 months, and neither vaccine was effective for treating horses that had been infected for more than 2 months.

The CMV vaccine consists of an extract of *P. insidiosum* prepared from disrupted *P. insidiosum* cells that had been desiccated. Mixed intracellular proteins are present in the CMV vaccine. In contrast, the SCAV vaccine comprises soluble mixed proteins precipitated from *P. insidiosum* cultures that had been concentrated by ultrafiltration. The SCAV vaccine contains mixed extracellular proteins.

The instant application describes and claims a method for preparing a vaccine that comprises an admixture of mixed extracellular and mixed intracellular proteins from *P. insidiosum* which have been isolated. Preferably the unwanted proteins and other compounds with a molecular weight less than 10,000 daltons are removed (Claims 26 and 27). To form the claimed vaccine, the claimed method combines two supernatants. The first supernatant containing the mixed extracellular proteins is an important component in the vaccine and its removal at one step in the method enables the mixed intracellular proteins to be effectively recovered from the cell mass in a smaller liquid volume. Therefore, preparing and

using a vaccine which combines (1) a product similar to the product of the CMV vaccine which contains mixed intracellular proteins with (2) a product similar to the product of the SCAV vaccine which contains mixed extracellular proteins, preferably followed by the important step of dialysis would not have been obvious to one skilled in the art.

The method using a vaccine as claimed that has the enhanced properties of the vaccine of the instant application would not be obvious to one skilled in the art. This is because both the CMV and the SCAV vaccines were equivalent in the efficacy. The equivalency in efficacy would not have led one skilled in the art to anticipate that a vaccine that combined mixed proteins supernatants would have resulted in a vaccine with enhanced curative properties. One skilled in the art would not be able to predict that the claimed method would provide a vaccine that was of enhanced efficacy.

One skilled in the art reading the Mendoza et al publications would realize that the SCAV vaccine containing the mixed extracellular proteins was no more effective than the CMV vaccine containing the mixed intracellular proteins. Neither reference provides any insight that the vaccine of the claimed method had enhanced efficacy over the prior art vaccines.

The claimed method provides a vaccine with therapeutic characteristics that are more effective in immunotherapy than either the CMV or SCAV vaccines of the

references. The claimed vaccine has remarkably enhanced curative properties and is able to cure horses that have been chronically infected with *P. insidiosum* for greater than 60 days (Specification - page 8, lines 22-27). The provided vaccine also cures all horses that had acute cases of *P. insidiosum* (Specification - page 8, lines 32-33). The prior art vaccines were only effective against *P. insidiosum* in horses infected less than 15 days and marginally effective in horses infected more than 45 days (Mendoza et al (AI)). Finally, the applicant also provides in Example 4 of the specification the remarkable ability of the provided vaccine to cure a human who had been infected with *P. insidiosum* for over 60 days prior to treatment with the provided vaccine. The enhanced properties of the vaccine provided by the claimed method of the instant application is clearly distinct from the prior art vaccines.

Therefore, while the references teach a need for a vaccine to treat *P. insidiosum* and provide several examples, the references do not teach or suggest the claimed method used the mixed extracellular and mixed intracellular protein vaccine. Merely stating a need exists to solve a problem does not then render any subsequent solution to the problem obvious to one skilled in the art.

The claims have been amended to clearly distinguish the present invention from Mendoza (IDS AI 1992) as discussed above. Nowhere in the prior art is

there a description of the use of the combined mixed extracellular and mixed intracellular proteins in a vaccine as claimed. Reconsideration is requested.

Claims 16 to 25 were rejected under 35 USC 103(a) as being unpatentable over Mendoza et al (1996). This reference does not describe the combined mixed extracellular and mixed intracellular proteins as now claimed for the same reason discussed in connection with Mendoza (AI; 1992). Certainly there is no expectation that the vaccine would be useful in humans or in animals. The results achieved with the claimed vaccine were very unexpected. This rejection is merely a hindsight reconstruction of the invention from Applicant's own disclosure, which is not permitted. Reconsideration of this rejection is requested.

It is now believed that Claims 16 to 27 are in condition for allowance. Notice of Allowance is requested.

Respectfully,

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Enclosure: Declaration of Biological Culture Deposit